Oncogenes

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Oncogene

- An oncogene is a mutated form of a normal cellular gene – called a *proto-oncogene* – that contributes to the development of a cancer.
- Proto-oncogenes typically regulate cell growth and cell differentiation. Most proto-oncogenes are highly conserved in evolutionarily diverse species, underscoring the fact that genes of this class play central roles in fundamental cellular processes. Mutations of protooncogenes that cause their conversion to oncogenes cause many of the perturbations in cell growth and differentiation that are commonly seen in cancer cells.

- An oncogene is a type of cancer gene. While all cancer genes are created by mutation, oncogenes are unique in that they are caused by mutations that alter, but do not eliminate, the functions of the proteins they encode. Proteins encoded by oncogenes typically show an increased level of biochemical function as compared with the protein products of the corresponding, nonmutated proto-oncogene.

- In 1908, Willhelm Ellerman and Olaf Bang demonstrated that a filtered extract devoid of cells and bacteria could transmit leukemia between chickens.

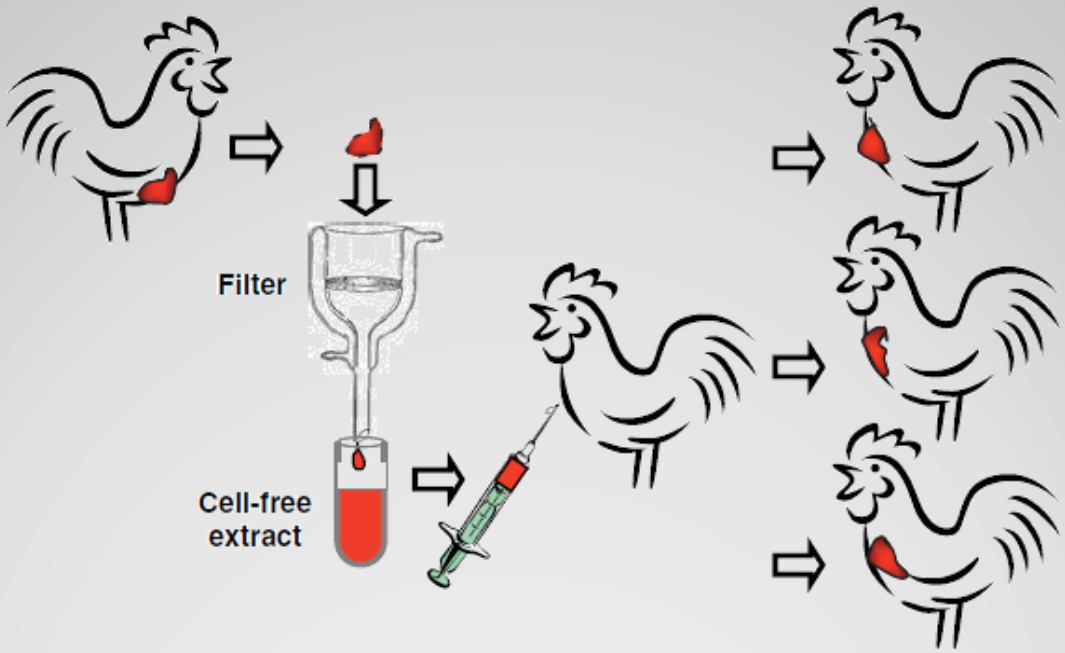


Fig. 2.1 The Rous experiment. A chicken sarcoma extract is prepared by filtration of a homogenized tumor (red). Injection of the cell-free filtrate results in horizontal transfer of the sarcoma to multiple chickens. This experiment demonstrated the infectious nature of this avian cancer

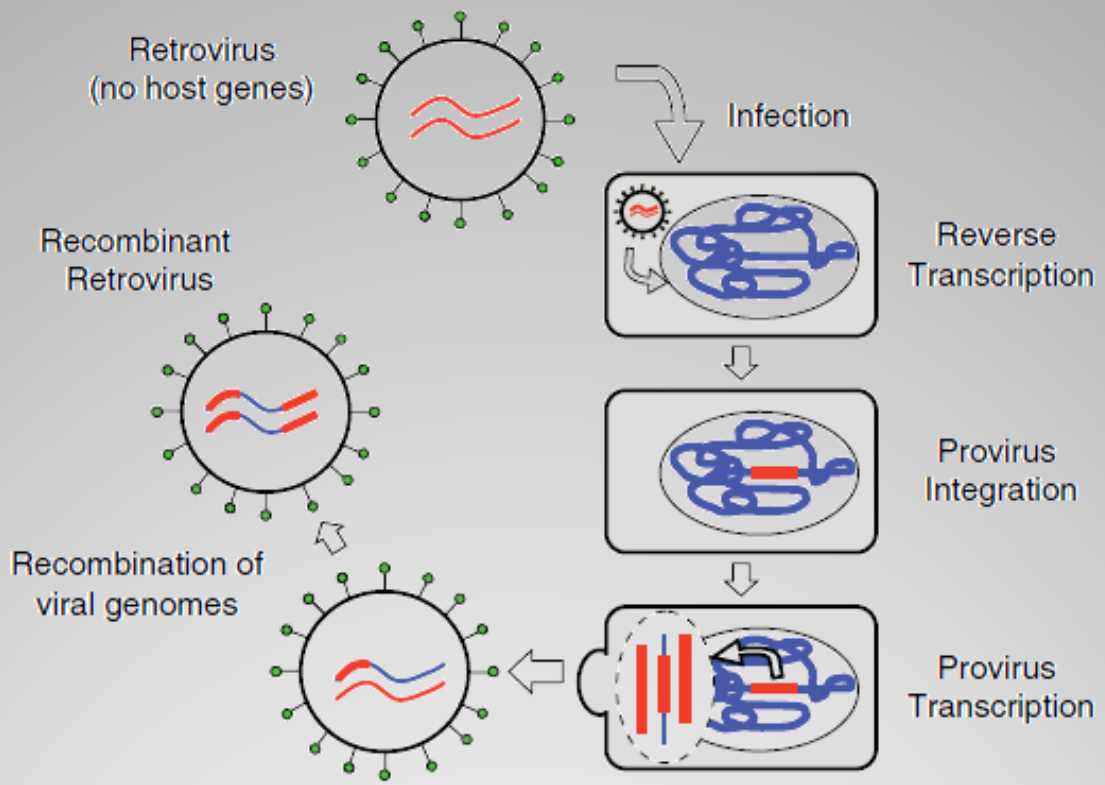


Fig. 2.2 The acquisition of oncogenes by retroviruses. The retrovirus capsule contains two copies of the viral RNA genome. After infection, the viral genome is copied into DNA by reverse transcriptase and integrates into the cellular genome as a provirus. If the provirus is integrated in close proximity to exon sequences, proviral transcripts can be spliced with host cell exons. These hybrid transcripts are packaged into a virion, resulting in a heterozygous viral genome. The viral genome undergoes recombination during a second round of infection. The resulting recombinant virus contains coding genetic elements that originated in the host cell

- Retroviruses can cause cancer in two different ways (Depending upon where they integrate, proviruses can disrupt the functions of host genes, usually by altering their transcriptional regulation.):
- 1. *slowly transforming retroviruses*: a proto-oncogene can be changed into an oncogene upon integration of a provirus. Typically, cancers caused by the disruption of a host gene by a provirus have a long latent period and take a long time to develop.
- 2. acutely transforming retroviruses such as RSV carry their own cancer genes.

- RSV contains a cancer gene known as SRC (pronounced ‘sark’). The protein encoded by SRC is an enzyme that localizes near the cell membrane and covalently modifies proteins in response to growth signals. Specifically, SRC encodes a protein tyrosine kinase, a class of enzymes that catalyzes the addition of a phosphate group onto the tyrosine residues of multiple protein substrates, thereby altering their function. Each covalent modification catalyzed by the SRC-encoded protein is one event of a series of enzymatically controlled events that collectively function to mediate signals that promote cell growth and division. In short, the SRC-encoded protein signals the cell to grow.

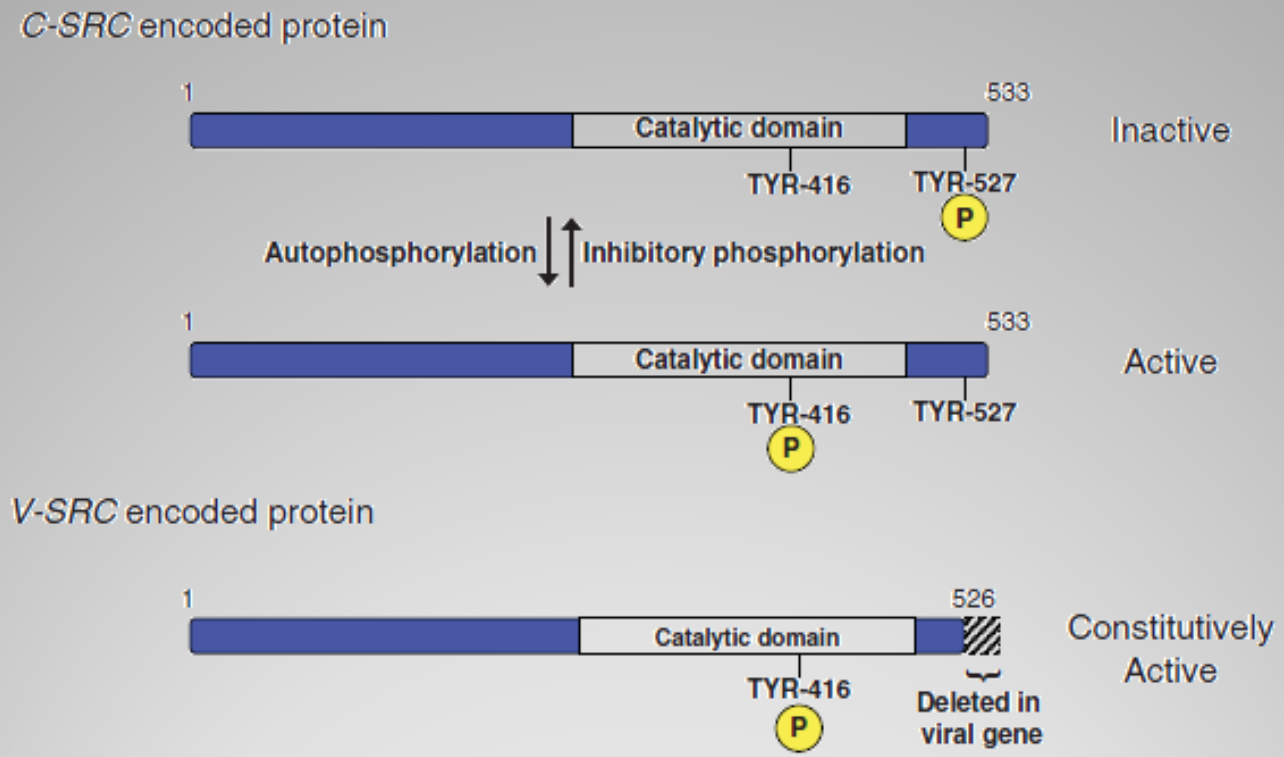


Fig. 2.3 Viral and cellular *SRC* genes. Cellular *SRC* (*C-SRC*) is a protein tyrosine kinase, 533 amino acid in length. Tyrosine autophosphorylation at residue 416 within the kinase domain causes a conformational change in the protein that results in the activation of kinase activity. Phosphorylation at tyrosine 527 by upstream inhibitory kinases prevents *C-SRC*-encoded protein activation. The viral oncogene *V-SRC* does not encode the c-terminal seven amino acids, and therefore does not contain the negative regulatory element

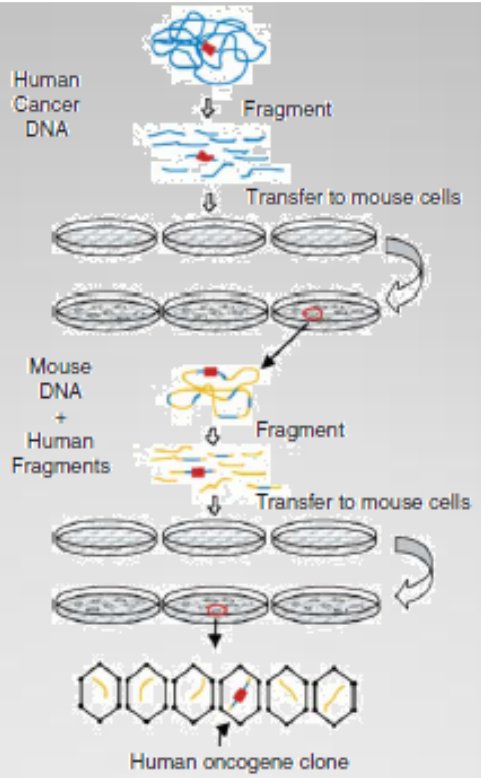
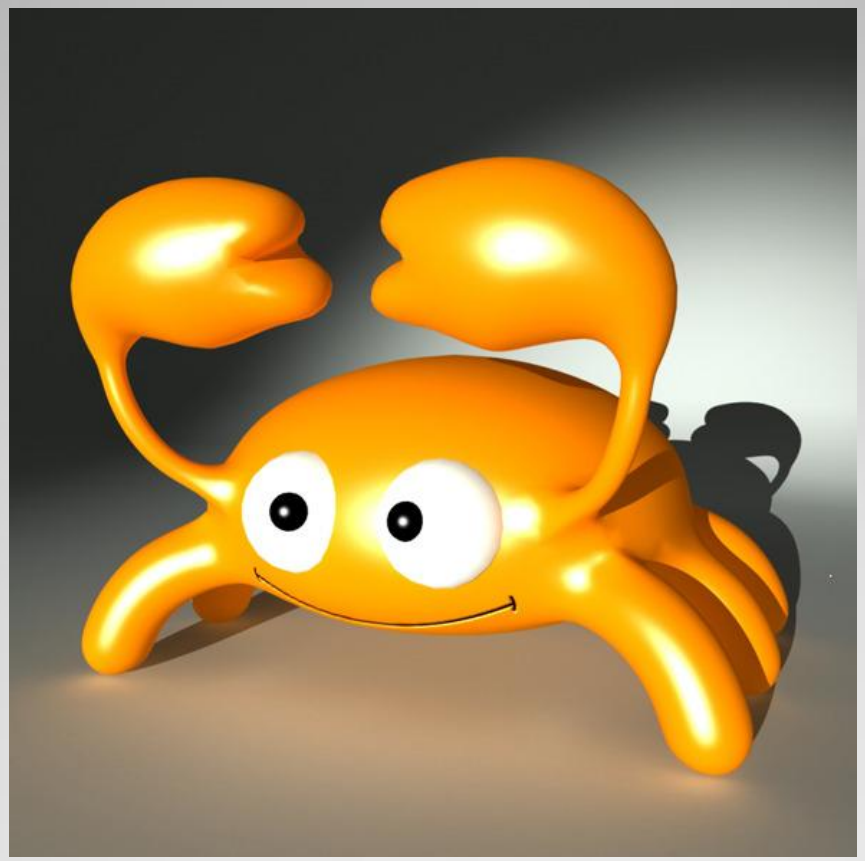


Fig. 2.4 Oncogene discovery by *in vitro* transformation. Genes transferred from human genomic DNA (blue) can alter the growth properties of mouse fibroblasts. Genomic DNA is sheared into smaller fragments, which are introduced into mouse cells growing in monolayer cultures. Appearing after a period of growth, discrete foci represent clones of mouse cells that have altered growth and cell–cell interactions. Genomic DNA from these clones (yellow) can contain multiple integrated fragments of human DNA. A second round of transfer allows the isolation of individual human fragments. DNA from the second clone is packaged into a bacteriophage library, which is then screened with a probe corresponding to human genomic DNA-specific repeat elements. Assays of this type were relatively nonspecific. Foci can be caused by actual oncogenes that are activated in cancer cells, but also by proto-oncogenes activated by the gene transfer process and growth regulatory genes that are not found to be mutated in cancers

The Search for Activated Oncogenes: The RAS Gene Family

Table 2.1 Mutations in the *RAS* gene family

Cancer type	Mutation frequency (%)	RAS family member
Pancreatic carcinoma	95	*K-RAS*
Colorectal carcinoma	50	*K-RAS*
Lung carcinoma	30	*K-RAS*
Acute Myelogenous Leukemia	25	*N-RAS*
Melanoma	10	*N-RAS*



Proto-Oncogene Activation by Gene Amplification

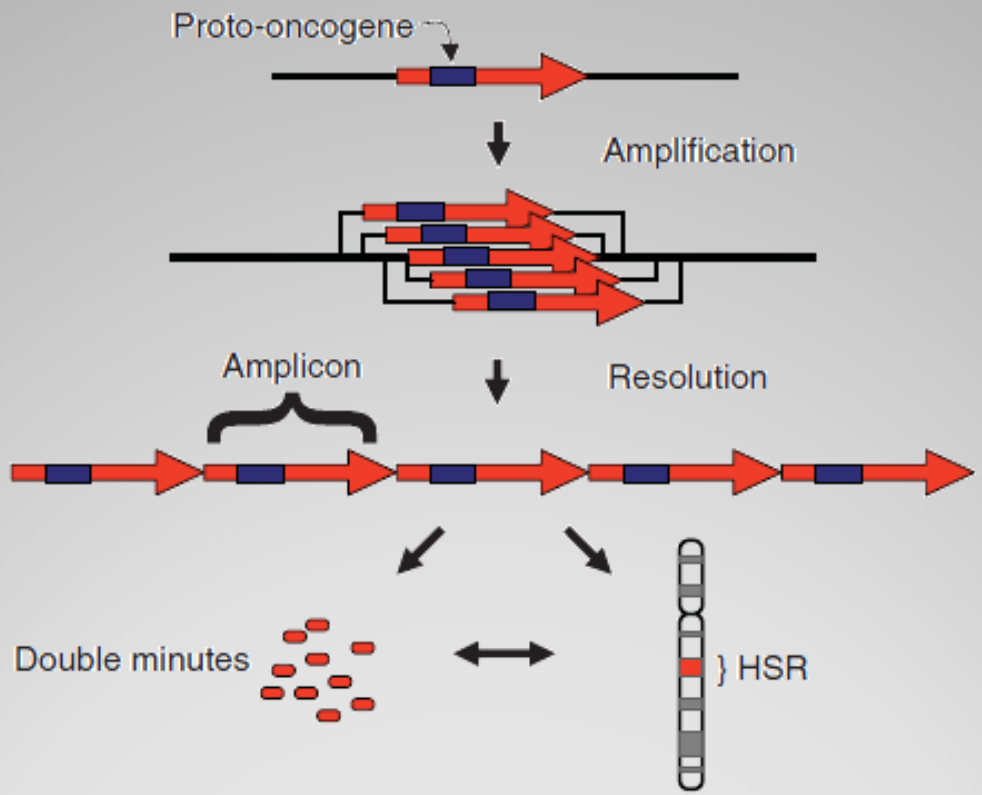


Fig. 2.5 Oncogene activation by gene amplification. A genomic region (red arrow) containing a proto-oncogene is amplified as a result of multiple rounds of DNA replication during a single cell cycle. Resolution of the over-replicated region results in a tandem array of amplicons in head-to-tail orientation. The amplified region can alternatively be maintained as double minutes, or integrated into a chromosome to form a heterogenous staining region (HSR). It is believed that these two configurations are interchangeable

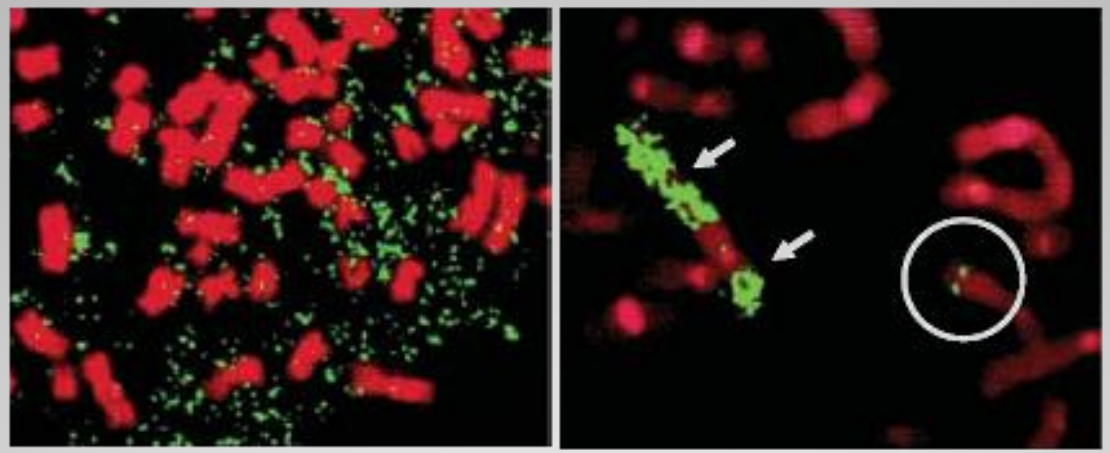
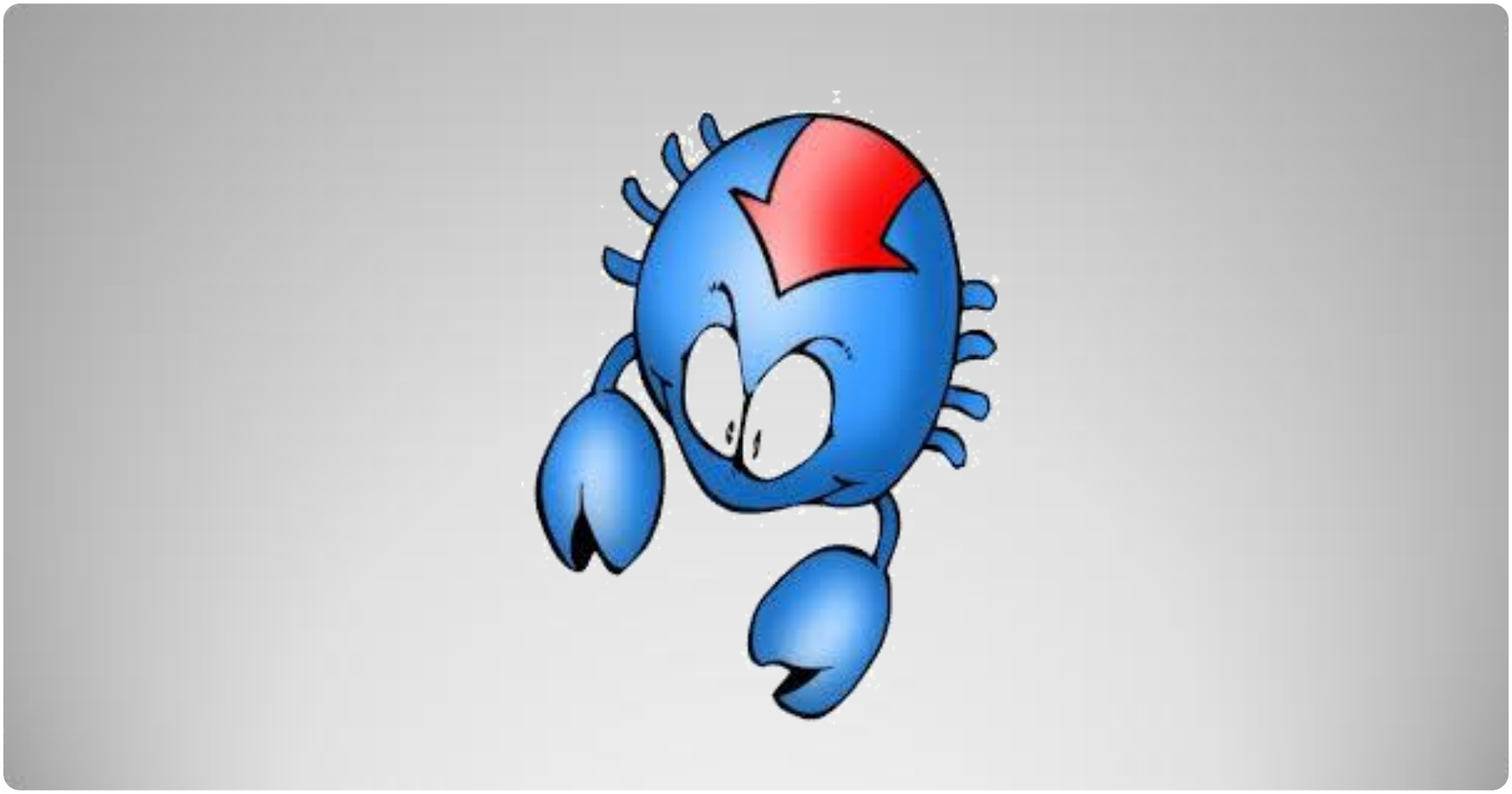


Fig. 2.6 Amplified C-MYC. The *MYC* locus in mitotic cells is stained green by fluorescence in situ hybridization. Shown as left are double minutes containing the amplified *C-MYC* locus. In the right panel are two homologously staining regions, indicated by arrows. Circled in the same panel are the two endogenous, unamplified *C-MYC* loci. (From Savalyeva and Schwab, *Cancer Lett.* **167**, 115–123 (2001). With permission.)

Table 2.2 Oncogenes frequently amplified in human cancers

Oncogene	Cellular function	Type of cancer	%
C-MYC	Transcription factor	Cervical	25–40
		Esophageal	38
		Breast	20
		Non-small cell lung	15
CCND1	Cell cycle regulator	Head and neck	50
		Breast	20
		Esophageal	25
		Hepatocellular	13
CCNE	Cell cycle regulator	Gastric	15
CDK4	Cell cycle regulator	Sarcoma	11–80*
		Glioblastoma	15
EGFR (ERBB1)	Growth factor receptor	Glioblastoma	33–50
ERBB2	Growth factor receptor	Medulloblastoma	40
(HER2/neu)		Breast	20–35
		Ovarian	20
		Cervical	20
		Non-small cell lung	10
HDM2	Regulation of tumor suppressor protein	Sarcoma	10–90*
MET	Protein tyrosine kinase	Esophageal	80
		Medulloblastoma	40
		Gastric	10–20
MITF	Transcription factor	Melanoma	20
PIK3CA	Lipid kinase	Medulloblastoma	45
		Ovarian	15

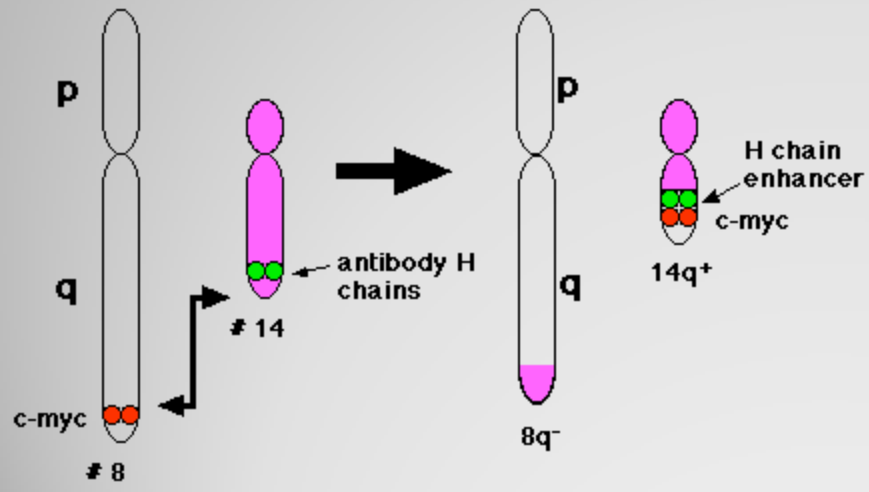
*Varies depending on cell type of origin.



Proto-Oncogene Activation by Chromosomal Translocation

T (8;14)

- An example of a proto-oncogene that can be activated by chromosomal translocation is C-MYC. The expression of C-MYC is normally tightly regulated. This tight transcriptional control is altered in some lymphomas and leukemia in which the C-MYC gene is repositioned, via translocation, into the vicinity of a highly active promoter. The repositioning of C-MYC into the vicinity of these strong promoters is sufficient to activate C-MYC, and thereby convert it into a functional oncogene.



T(9;22)

- At the molecular level, the consequence of the translocation involving chromosomes 9 and 22, denoted t(9;22), is the unique juxtaposition of two genes, BCR and C-ABL. C-ABL is a proto-oncogene homologous to an oncogene originally found in the retroviral genome of the Ableson leukemia virus. In the absence of translocation, the expression of the C-ABL proto-oncogene is tightly regulated. The BCR gene, in contrast, was so named because of its location within the break point cluster region on chromosome 22. BCR expression is driven by a strong, constitutively active promoter. Strictly speaking, BCR is not considered a proto-oncogene, and in fact its normal cellular role is unknown. The BCR promoter functions to transcribe C-ABL exons when the two genes are fused by translocation

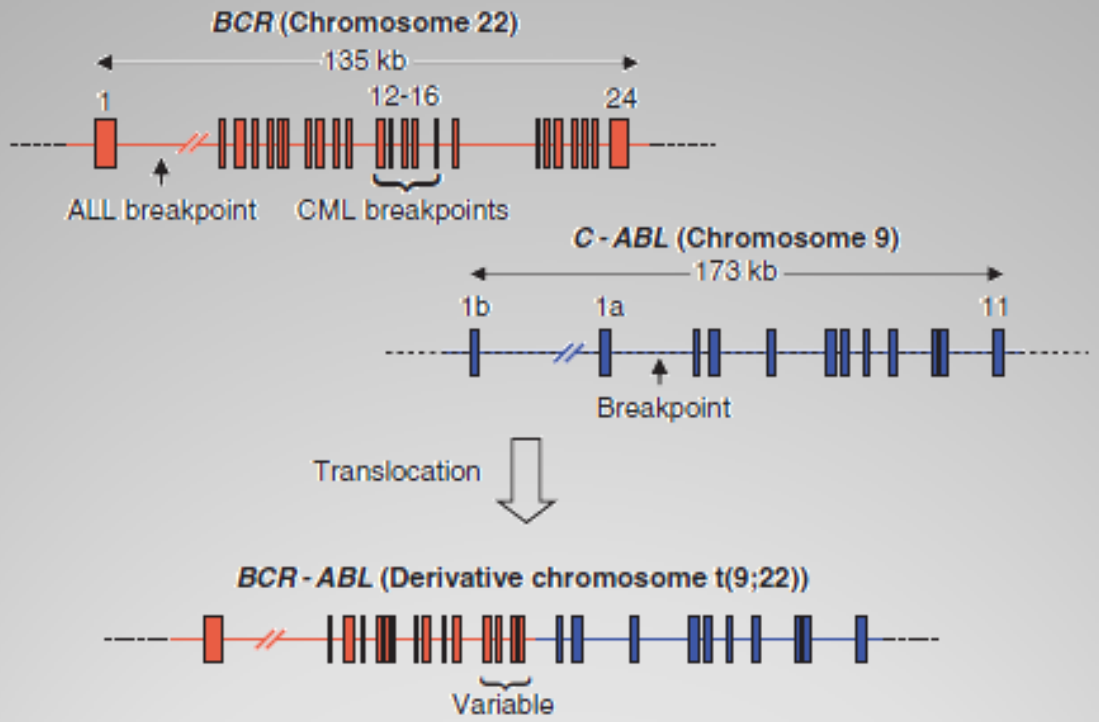


Fig. 2.8 The creation of *BCR-ABL* by translocation. The BCR locus on chromosome 22 spans roughly 135 kb and is composed of 24 exons. Within this gene is a recurring break point found in acute lymphocytic leukemia-associated translocations, and a cluster of break points found in chronic myeloid leukemias. The C-ABL locus on chromosome 9 spans 173 kb and has 11 exons. Note that there are two first exons that are alternatively utilized. A single recurrent break point occurs upstream of exon 2. In the t(9;22) derivative, the *BCR* and *C-ABL* genes are fused, and contain a single open reading frame. The different CML-associated break points in *BCR* result in the variable inclusion of *BCR* exons 12–15 in different allelic forms of *BCR-ABL*

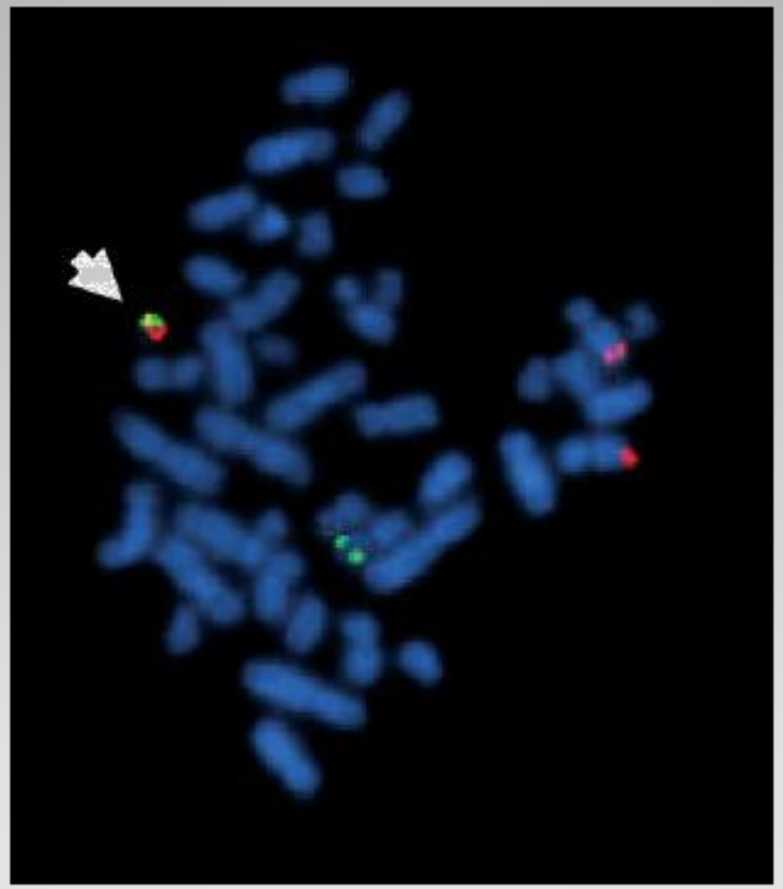


Fig. 2.7 The Philadelphia chromosome. The Philadelphia chromosome (indicated by arrow) stained during mitosis. Fluorescence in situ hybridization probes are derived from *BCR* (green) and *C-ABL* (red). The spots in other chromosomes represent the untranslocated *BCR* and *C-ABL* genes

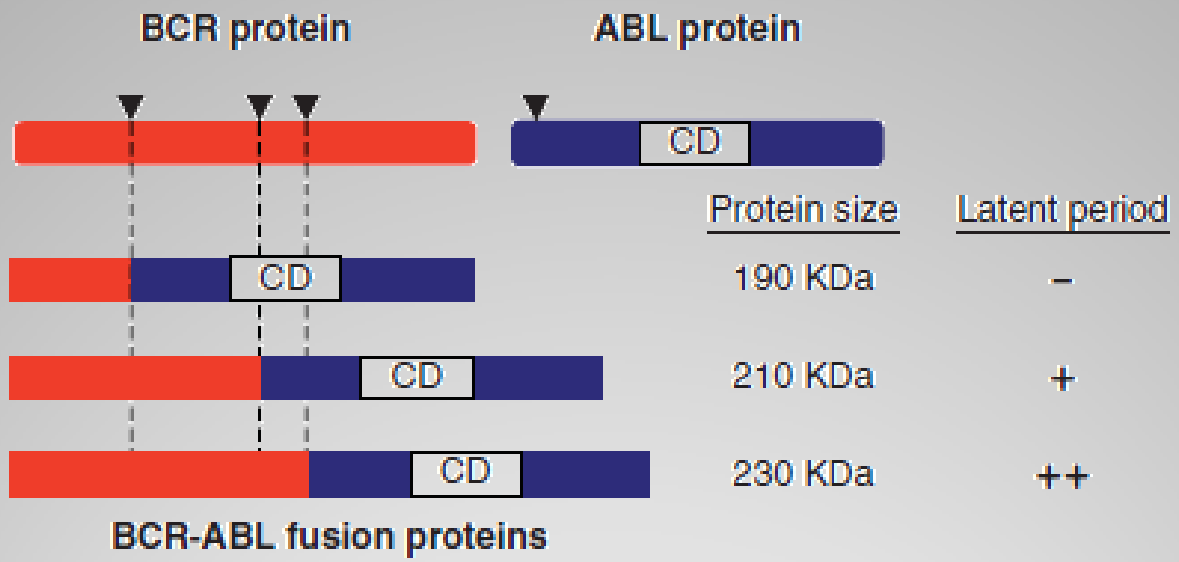
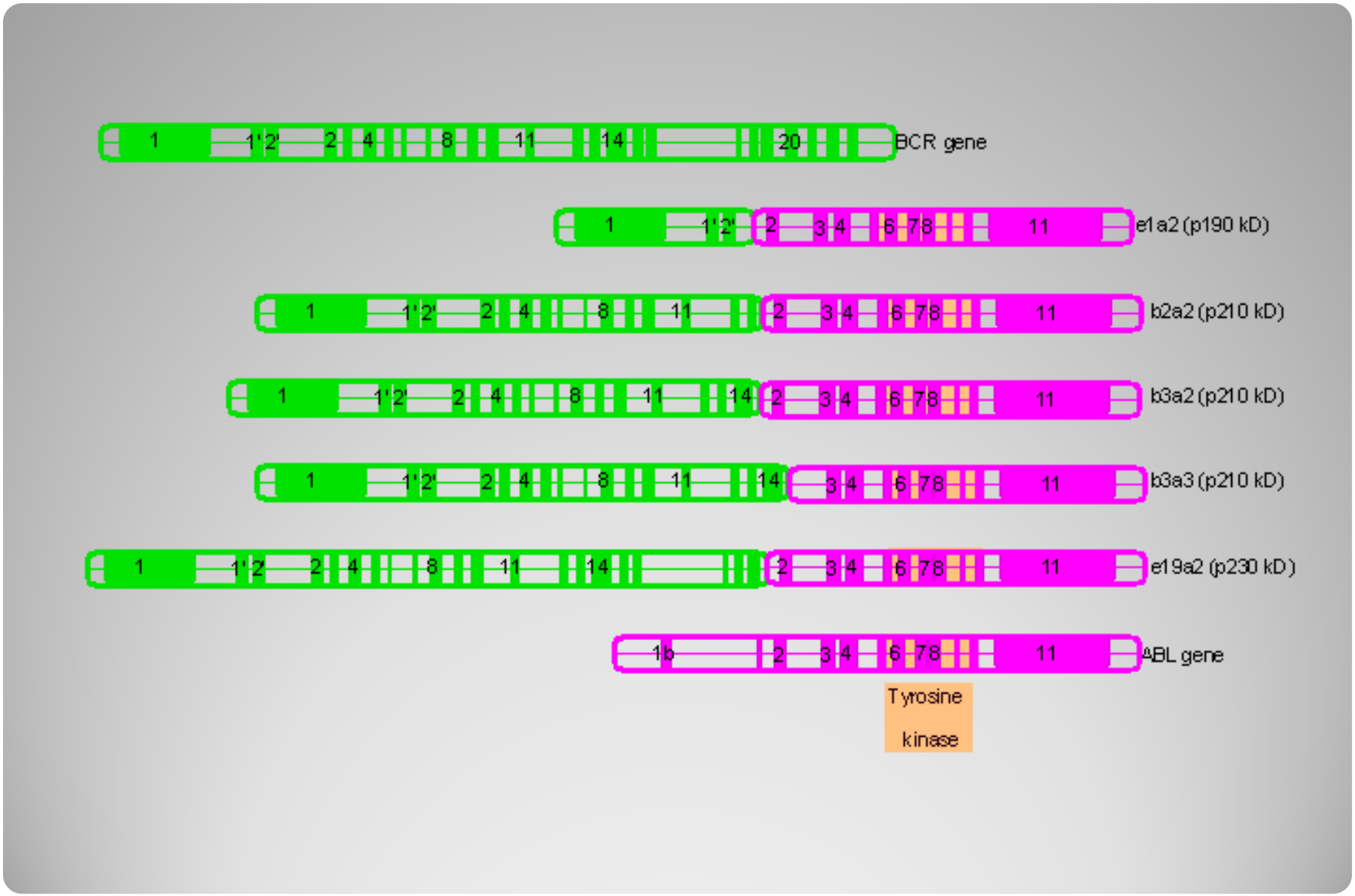


Fig. 2.9 *BCR-ABL*-encoded proteins. The primary structures of the native BCR and ABL proteins are shown. Arrowheads indicate the regions of defined by the recurrent break points. The various break points in *BCR* lead to the appearance of distinct fusion proteins with molecular weights of 190, 210 and 230 kDa. The 190 KDa protein is restricted to ALL, an acute disease that is not characterized by a latent period. The 210 KDa is the most prevalent CML-associated version. while the 230 KDa protein is found in a subset of CML patients that typically exhibit an extended period of disease latency


1 1' 2' 2 4 8 11 14 20
BCR gene
1 1' 2' 2 3 4 6 7 8 11
e1a2 (p190 kD)
1 1' 2' 2 4 8 11 2 3 4 6 7 8 11
b2a2 (p210 kD)
1 1' 2' 2 4 8 11 14 2 3 4 6 7 8 11
b3a2 (p210 kD)
1 1' 2' 2 4 8 11 14 3 4 6 7 8 11
b3a3 (p210 kD)
1 1' 2' 2 4 8 11 14 2 3 4 6 7 8 11
e19a2 (p230 kD)
1b 2 3 4 6 7 8 11
ABL gene
Tyrosine kinase

- Acute promyelocytic leukemia (APL or AML-M3) is a subtype of acute myeloid leukemia with distinct clinical and histopathologic features. Historically one of the most lethal forms of acute myeloid leukemia, APL leads to disseminated intravascular coagulation and death when not diagnosed and treated. Treatment with all-trans-retinoic acid substantially improves survival in patients who have failed anthracycline chemotherapy or for whom anthracycline is contraindicated. Similarly, arsenic trioxide is beneficial in APL patients who have failed or have contraindications for treatment with anthracycline or retinoid-based therapy.
- Genetically, APL is characterized by a unique chromosomal anomaly. More than 99% of APL patients harbor a translocation between chromosomes 15 and 17, which fuses the retinoic acid receptor alpha (RARA) gene on chromosome 17 with the PML gene on chromosome 15. A short or a long transcript isoform is produced, depending on the PML breakpoint; the short isoform has been linked to poor outcome, but this association remains controversial. Detection of the PML/RARA t(15;17) translocation is diagnostic for APL, although the diagnosis can also be based on morphology. The presence of this translocation is necessary for response to all-trans-retinoic acid and arsenic trioxide. Thus, the PML/RARAt(15;17) assay is useful for diagnosis and predicting treatment response. It is also helpful for monitoring therapeutic response and MRD and for detecting early relapse.

PML/RARA t(15;17) Translocation

Clinical Use

- Diagnose acute promyelocytic leukemia (APL)
- Predict response to all-trans-retinoic acid or arsenic trioxide therapy
- Assess effectiveness of therapy
- Monitor minimal residual disease (MRD)
- Predict early relapse

- As we have seen throughout this chapter, a single mutation is sufficient to activate a proto-oncogene and convert it to an oncogene. The activating mutation results in a growth advantage, in spite of the continued presence of a normal, unmutated allele in every cell. Because the phenotype conferred by an oncogenic mutation is not masked by the presence of the remaining wild type allele, oncogenes are, by definition, dominant alleles.

Oncogenes are Dominant Cancer Genes